Title	STUDIES OF JAPANESE COMPOUND STYELID ASCIDIANS. -III. A NEW, POSSIBLY ASEXUAL POLYANDROCARPA FROM SHIMODA BAY-
Author(s)	Kawamura, Kazuo; Watanabe, Hiroshi
Citation	PUBLICATIONS OF THE SETO MARINE BIOLOGICAL LABORATORY (1981), 26(4-6): 425-436
Issue Date	1981-09-30
URL	http://hdl.handle.net/2433/176029
Right	
Type	Departmental Bulletin Paper
Textversion	publisher

STUDIES OF JAPANESE COMPOUND STYELID ASCIDIANS. III. A NEW, POSSIBLY ASEXUAL *POLYANDROCARPA* FROM SHIMODA BAY[1)]

KAZUO KAWAMURA[2)] and HIROSHI WATANABE

Shimoda Marine Research Center, University of Tsukuba

With Text-figures 1–8

So far, only three species of the genus *Polyandrocarpa* have been reported from the Japanese waters (Tokioka, 1953; Watanabe and Tokioka, 1972). The genus *Polyandrocarpa* shares the same structural characteristics with *Polycarpa*, but the former is separable from the latter only by the asexual reproduction carried out in the former by means of palleal budding. In summer, 1978, colonies of a strange compound ascidian, possibly of the genus *Polyandrocarpa* were found attached on the test of *Halocynthia ritteri* (Oka) collected from Shimoda Bay. Since then, these colonies have been reared continuously on glass slides kept in culture boxes set in the inlet adjoining the Shimoda Marine Research Center, University of Tsukuba, and the external and internal structures of zooids were observed in detail every one or two days as well as their budding and bud development.

Careful observations on the colonies, cultured for 2 years and 3 months, have revealed some very noteworthy features that might present some significant interpretation in the taxonomy of compound styelids in future. These are the irregular arrangement of stigmata, the complete absence of any sign of gonad, and the peculiar feature of asexual reproduction by stolonic budding. Especially, non-appearance of gonads during the observations has made the generic attribution of the present polystyelid quite difficult. Fortunately, some live colonies of *Polyandrocarpa* (*Eusynstyela*) *misakiensis* Watanabe and Tokioka, 1972 were still available at the Research Center. So, the arrangement of the test-vessels and orifices and the development of the adult stigmatal arrangement as well as the mode of stolonic budding were compared with each other in detail between the present polystyelid and *P. misakiensis*. Encouraged, then, by close resemblance between these two forms in the aspects mentioned above, the authors have decided to describe the present form provisionally as a new species of *Polyandrocarpa* and to give here full explanation of its asexual reproduction and development of blastozooids in comparison with those in *P. misakiensis*.

1) Contributions from the Shimoda Marine Research Center, No. 386.

2) Present address: Department of Biology, Faculty of Science, Kochi University, Akebono-cho, Kochi 780.

Publ. Seto Mar. Biol. Lab., XXVI (4/6), 425–436, 1981. (Article 23)

Before going further, the authors want to present their sincere thanks to Dr. Takasi Tokioka, Professor Emeritus of Kyoto University, and Mr. Teruaki Nishikawa of the College of General Education, Nagoya University, for their suggestions and advices in taxonomy, especially to the former for his kindness in reading the manuscript critically and to the latter for his generosity to make observations of endocarps, atrial tentacles, ciliated groove, and inner longitudinal vessels for the authors. They are also deeply indebted to the members of the Shimoda Marine Research Center for their kind assistance.

Polyandrocarpa stolonifera n. sp.

Description: The colony consists of aggregated zooids that are independent of one another, without any functional connection among them (Fig. 1A). The arrangement of zooids in the colony is quite irregular. Fully grown-up zooids (Fig. 2) are oval in outline, about 5 mm in length, and seem reddish when alive through the test which is thin but hard gelatinous, translucent and colorless, and quite smooth on the surface. Only the periphery of the neural ganglion looks whitish for the nephrocytes accumulated there. Apertures are respectively subterminal on the dorsal side, each opened on a short siphon, and margined smoothly (Fig. 7 B and D). There are thirty or more small endcarps on the inner surface of mantle.

Fine atrial tentacles are present. There are 6 branchial tentacles, and sometimes

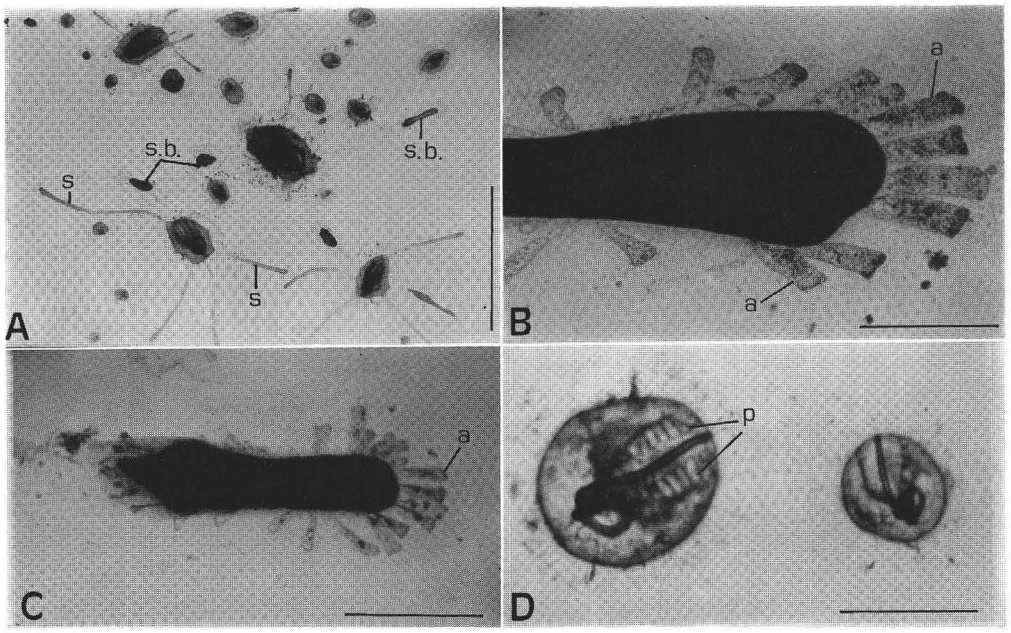

Fig. 1. A, a colony. Bar, 10 mm. B, a tip of stolon. Bar, 0.2 mm. C, a stolonic bud. Bar, 0.4 mm. D, developing blastozooids. Bar, 0.4 mm. All figures were photographed from the ventral side. a, ampullae; p, protostigmata; s, stolon; s. b., stolonic bud.

with a few minute papillae. The ciliated groove is a longitudinal slit. Of the four branchial folds on each side, the first (dorsal-most) fold and the third are well developed, while the second and the fourth are rather rudimentary, and especially the last, ventral-most one is confined to the anterior half of the branchial sac. Stigmata are generally elongated transversely, but rather irregular in both size and arrangement, though originally up to 9 stigmatal rows are definable on the left ventral side and a few to several stigmata found between the endostyle and the fourth fold are elongated antero-posteriorly (Fig. 2). Inner longitudinal vessels are arranged in the anterior region of the branchial sac as follows:

left side,	dorsal lamina	0	(7)	1	(3)	1	(9)	1	(6)	0 endostyle
right side,	dorsal lamina	0	(9)	1	(4)	1	(9)	1	(6)	0 endostyle

The visceral mass is situated roughly on the left posteroventral side of the body, though a half (in younger zooids) to a third (in larger zooids) of the stomach is extended onto the right side over the midventral line. The anterior edge of the intestinal loop attains anteriorly around the level of the posterior third of the branchial sac. The stomach is about half as long as the visceral mass, situated with its axis

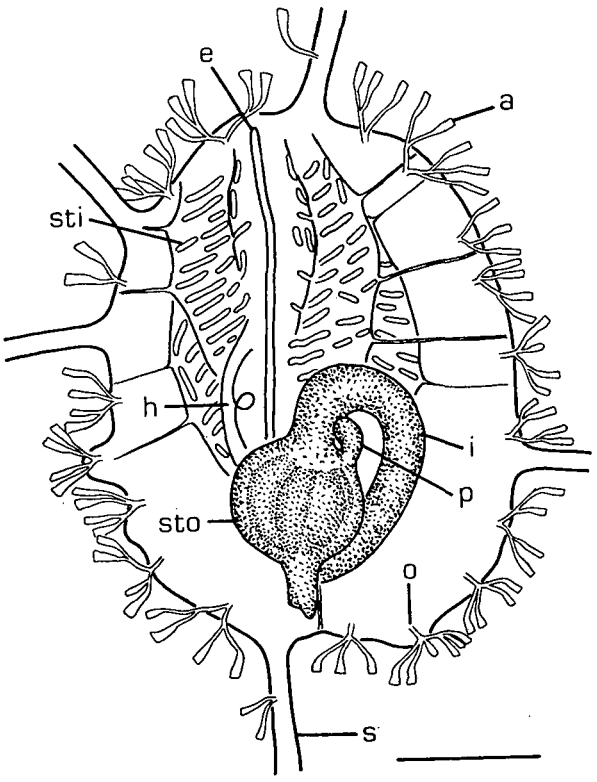

Fig. 2. An adult zooid, ventral view. Bar, 1 mm. a, ampullae; e, endostyle; h, heart; i, intesine; o, orifice; p, pyloric coecum; s, stolon; sti, stigmata; sto, stomach.

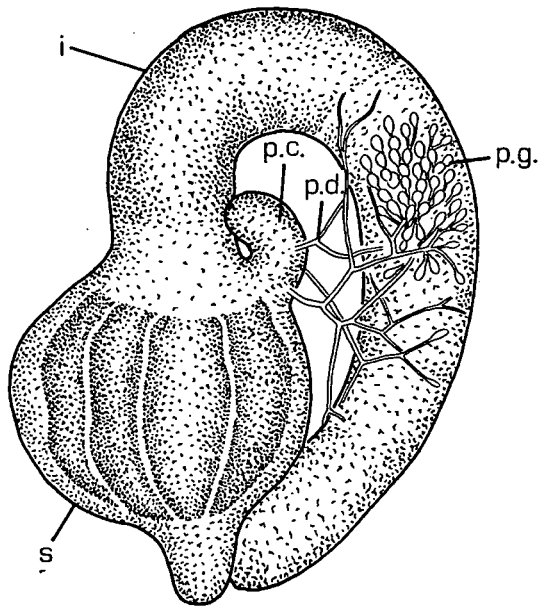

Fig. 3. The digestive tract, from the side of attachment. Bar, 0.5 mm. i, intestine; p.c., pyloric coecum; p.d., pyloric duct; p.g., pyloric gland; s, stomach.

parallel to the body axis, and furnished with 6–7 plications, not so well-marked, and the pyloric coecum, rather short and bent inwards. A few thick pyloric ducts are issued from the basal part of the coecum and leading to the circum-intestinal gland developed rather poorly on the intestine opposite the coecum across the space of the loop, after branching several times (Fig. 3). No gonads have been observed.

There are 15 to 18 orifices respectively leading to the test-vessels, on the wall of the peribranchial cavity in adult zooids. They are opened on the ventral side not so far apart from the basal margin (Figs. 2 and 6); the test vessels end each to vascular ampullae after branching a few to several times. Thus, the zooids are surrounded by many ampullae as in *P. misakiensis* (Fig. 8 C and D). The present polystyelid forms stolons (Fig. 1 A). The stolons may be produced from any places along the basal margin of zooids and usually are extended more than 10 mm in length, the longest stolon observed being 18 mm. Generally 1 to 3, rarely 5, stolonic buds are formed from respective stolons.

Bud formation and development: Anlagen of stolon are produced as a protrusion of the mantle wall (Fig. 1 A) and accompanied with vascular ampullae as in other polystyelid ascidians, though the ampullae are much fewer and mostly limited to the distal portion of stolon throughout the course of the stolon growth in the present species (Figs. 1 B and 4). As the orifices apart from the center of the stolon Anlage more than 0.2 mm are never introduced into the stolon, it is suggested that the parent tissue of 0.3–0.4 mm wide along the basal margin of the mantle will participate in

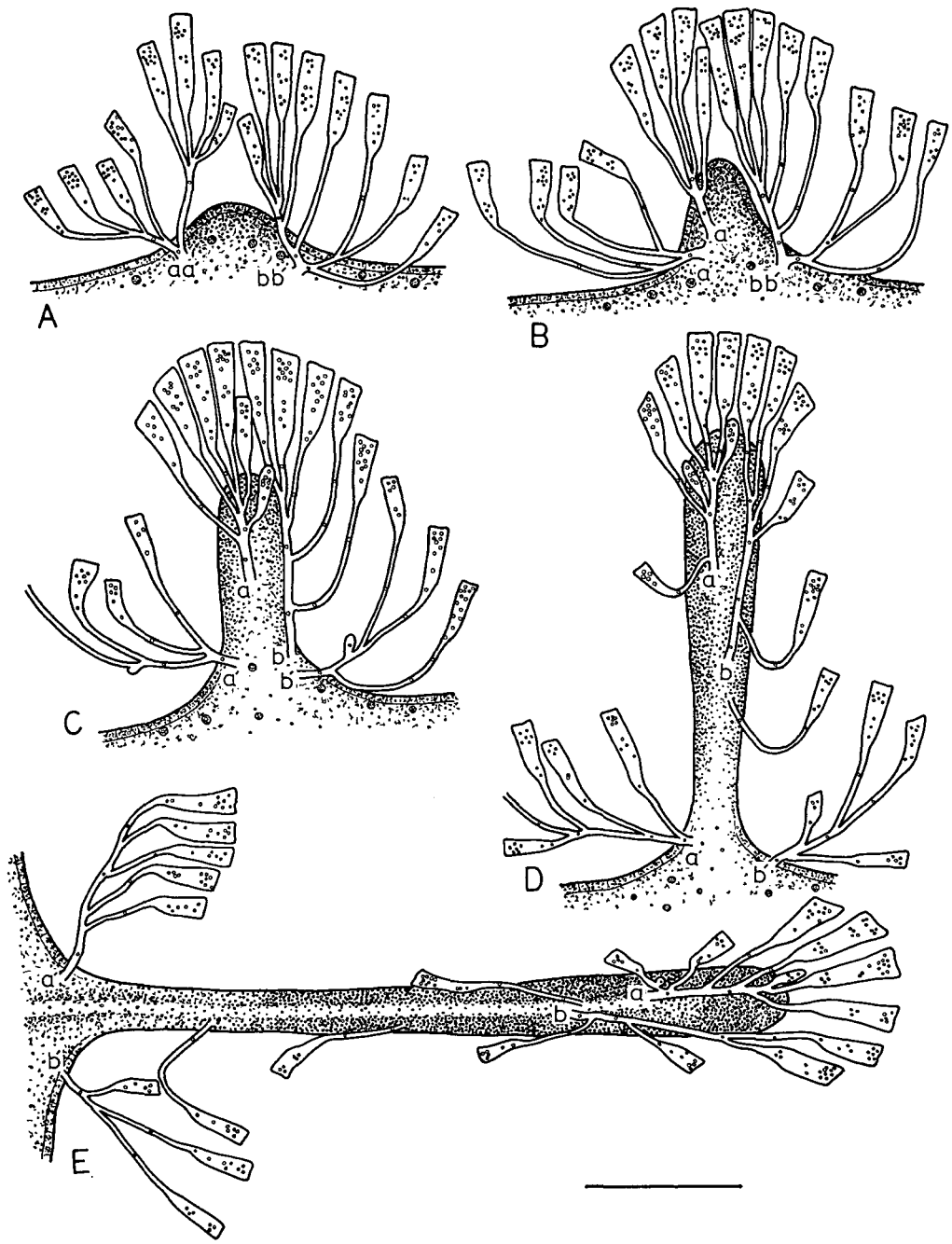

Fig. 4. Daily growth of a stolon, ventral view. Letters, a, a′, etc. each indicate the position of orifices. Bar, 0.5 mm.

the stolon formation. Usually one or two orifices are brought into the growing stolon and carried away near to the stolon tip in its growth. In the first one or two days of the growth of the stolon, the span between the stolon tip and the distal orifice will be extended to about 0.4 mm, but thereafter this span is maintained constant (Fig. 5). The orifices and then the test-vessels are thus moved distally with growth of the stolon, therefore no test-vessels are found running proximo-distally beneath the stolon.

Strobilation of the stolon takes place about a month after the protrusion of the stolon Anlage and after this, respective stolonic buds formed from the stolon are shrunk to an elongated elliptical body, the proximal half of which is slightly swollen and with rather irregular basal margin (Figs. 1 C and 6 A). Heart beating is observable about 3 days later (Fig. 6 B and C), and both branchial and atrial apertures are perforated and feeding starts 6 to 7 days later. Five to six protostigmata appear

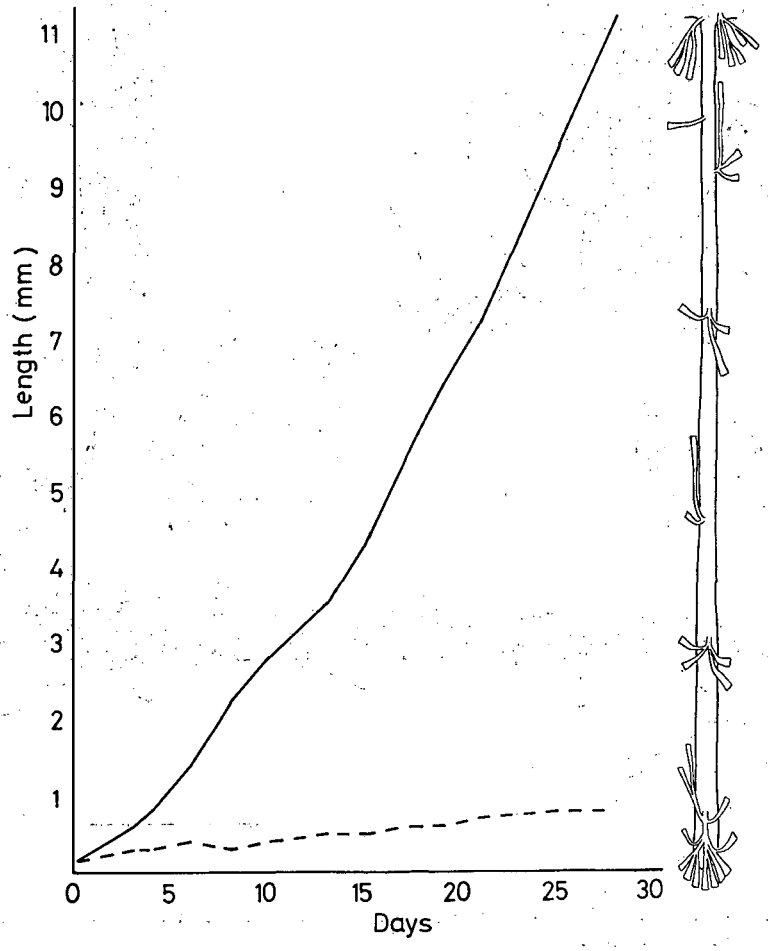

Fig. 5. Growth curve of a stolon. Broken line indicates the span between the stolon tip and the distal-most orifice.

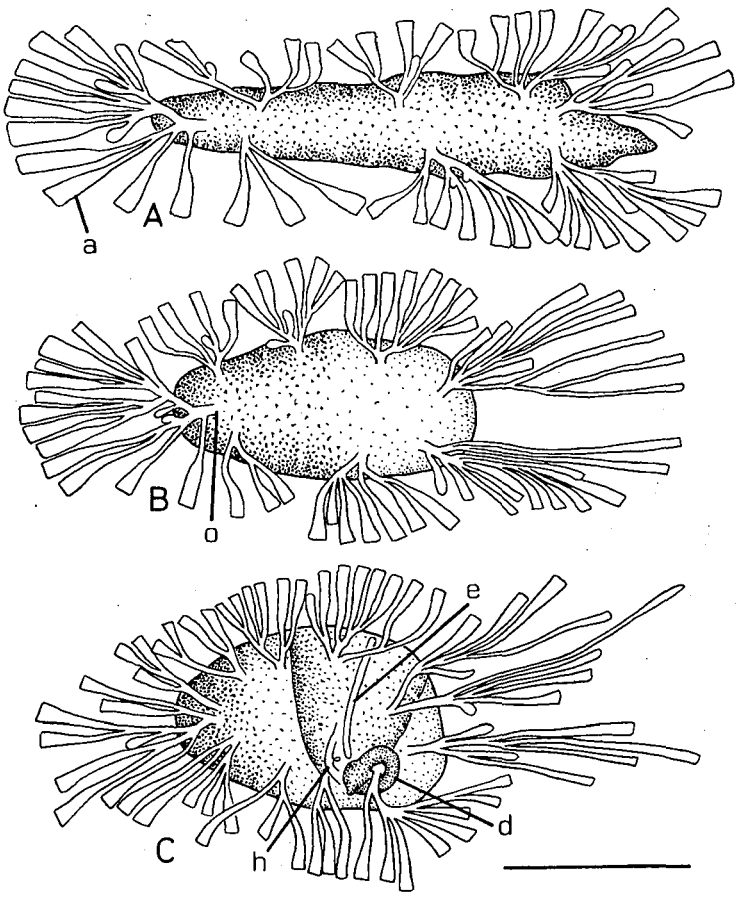

Fig. 6. Daily observation on the distal-most one of the 3 stolonic buds formed on a stolon, ventral view. Bar, 0.5 mm. a, ampullae; d, digestive loop; e, endostyle; h, heart; o, orifice.

on the plain wall of the branchial sac on the ventral side about 6 days later (Figs. 1 D and 7 A). They are not extended throughout the whole side of the branchial sac, but are each interrupted at the site corresponding to the second branchial fold and thus divided into dorsal and ventral halves. The first and the third folds are then formed and each half of respective protostigmata will be further subdivided at these folds. Thus, four longitudinal rows of stigmata elongated transversely are formed on each side of the branchial sac (Fig. 7 B, C, and D). Later, the fourth, ventral-most fold is formed in the anterior region, and a short row consisting of several stigmata only is formed on each side of the endostyle in the anterior part of the sac (Fig. 7 E).

By this stage, stigmatal rows are increased up to 9 at least on the left ventral side. Further division of stigmata takes place in places till the stigmata of an ordinary size, though elongated transversely, are produced. Only the stigmata between the endostyle and the fourth fold are often elongated longitudinally (see Fig. 2). As

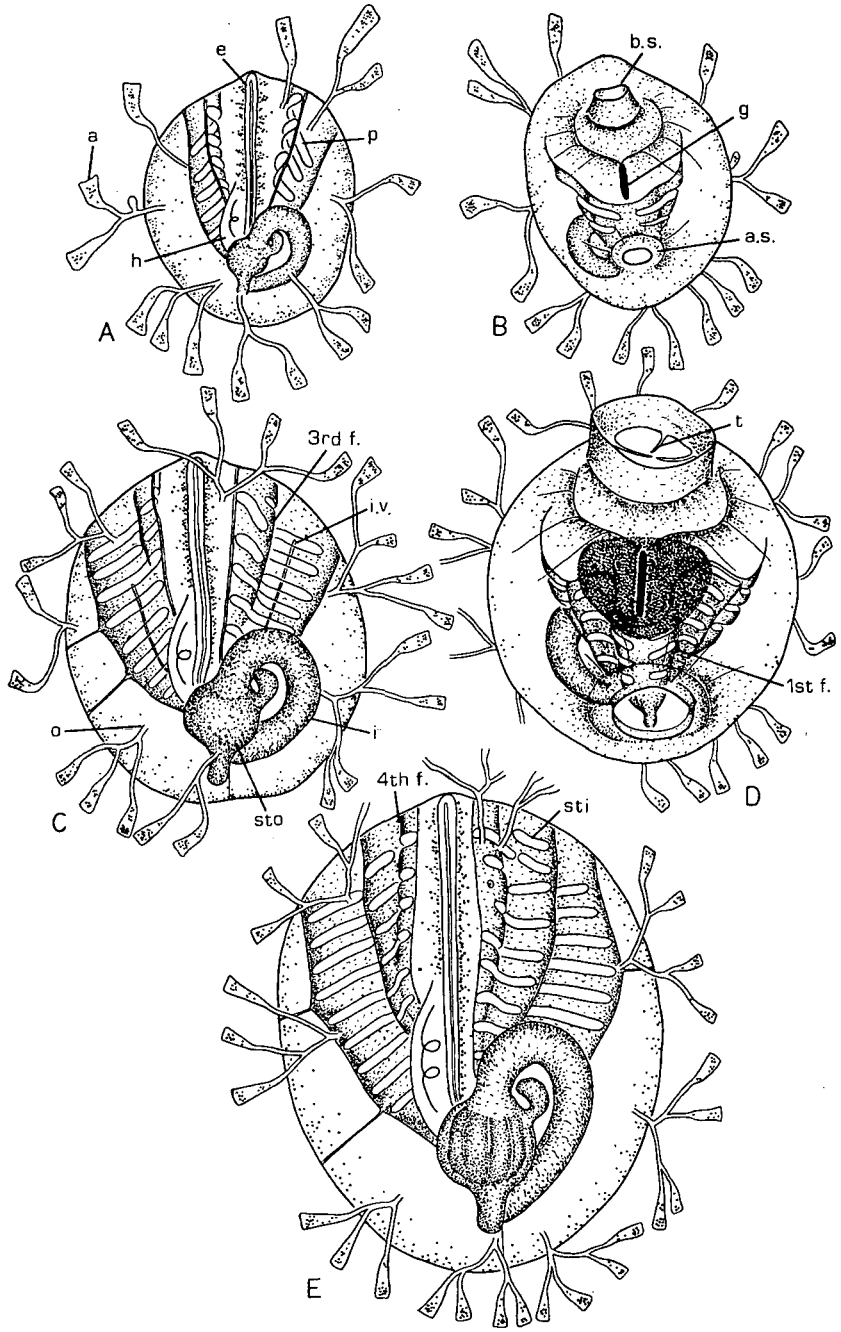

Fig. 7. Development of stolonic buds. A, 7-day old blastozooid, ventral view. B, the same zooid, dorsal view. C, 10-day old blastozooid, ventral view. The third branchial fold (3rd f.) is just being formed. D, the same, dorsal view. The first fold (1st f.) is formed already. E, 12-day old blastozooid, ventral view. The fourth fold (4th f.) is seen. Bar, 0.5 mm. a, ampullae; a.s., atrial siphon; b.s., branchial siphon; e, endostyle; g, ganglion; h, heart; i, intestine; i.v., inner longitudinal vessel; o, orifice; p, protostigmata; sti, stigmata; sto, stomach; t, tentacle.

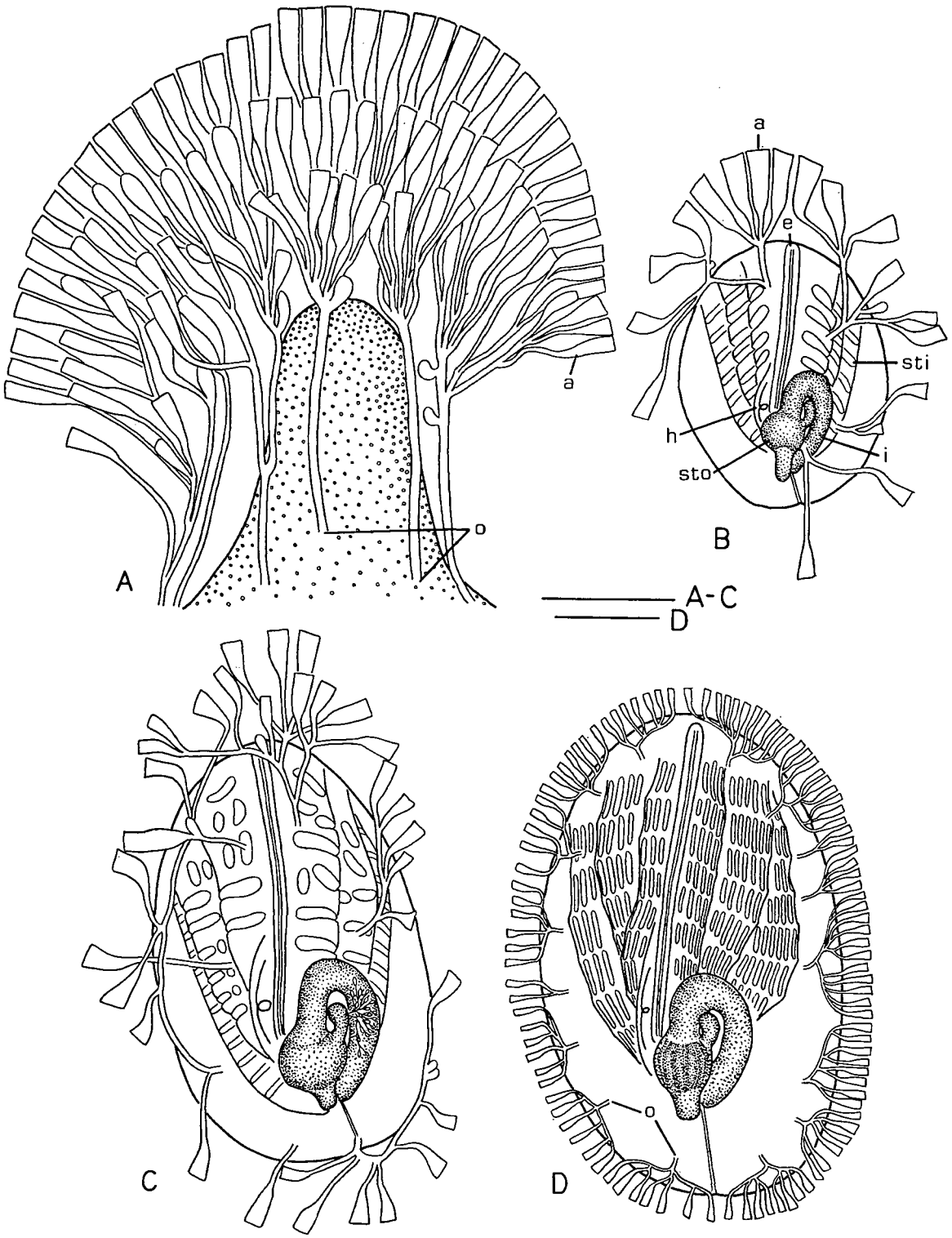

Fig. 8. Bud development and adult zooid of *P. misakiensis*, ventral view. A, a growing bud. B, a developing blastozooid. Transversely elongated stigmata are seen. C, a zooid further developed. Protostigmata are being subdivided. D, a young adult blastozooid. Bars indicate 0.5 mm in A-C and 1.0 mm in D, respectively. a, ampullae; e, endostyle; h, heart; i, intestine; o, orifice; sti, stigmata; sto, stomach.

the protostigmata found also in young blastozooids of *P. misakiensis* are divided successively till the branchial sac is wholly furnished with ordinary stigmata longitudinally elongated (Fig. 8 B, C, and D), the successive division of protostigmata in the present new species may be expressed as imperfect.

Considerations

The present species particularly resembles *Polyandrocarpa* (*Eusynstyela*) *transversalis* Tokioka, 1963 from Hawaii in the general structure of the branchial sac, though the ventral-most fold poorly developed in the former remains in the latter in the state of a group of several inner longitudinal vessels.

The test-vessels and their proximal points, or "the orifices", have been well investigated in some species of *Botryllus, Symplegma,* and *Metandrocarpa.* In *Botryllus,* zooids are provided with two types of vessels and orifices: One type is formed from bud stalks which connect zooids and buds of three generations, or a unit (Watanabe, 1953), with one another, and the other is formed as the outgrowth of the ventral epidermis of developing buds (Izzard, 1973), and this connects the zooids or buds with colonial circulatory system (Mukai *et al.*, 1978). In *Symplegma,* there are 3 orifices, the first on the posterior, the second on the left ventral side, and the third on the right ventral side (Sugimoto and Nakauchi, 1974; Kawamura and Nakauchi, 1976). In *Metandrocarpa,* 4–5 orifices are present approximately in the mid-ventral region and subzooidal tracts are developed in a dendritic pattern beneath each zooid (Newberry, 1965; Kawamura and Watanabe, 1981). In contrast with these, in the present new species, there are 15–18 orifices along the basal margin in adult blastozooids, but without any test-vessels underlying them. The same is seen in *P. misakiensis.* Nothing has been known about the orifices in *Stolonica* and *Distomus,* though Sélys-Longchamps (1917) and Berrill (1948) illustrated the test vessels running beneath the stolon or bud in *S. socialis* and *D. variolosus,* respectively. This feature resembles *Metandrocarpa* and *Polyzoa* rather than *Polyandrocarpa.* Thus, the similarity with *Polyandrocarpa* in the feature of orifices and test-vessels has led the authors to the present generic identification of the present species, though the subgeneric attribution is still unknown.

The formation of stolonic buds in the genus *Polyandrocarpa* is reported here for the first time. Almost nothing has been known how the component cells of the palleal bud are supplied. In this connection, it is noteworthy that the stolon in the present new species seems to be elongated without further supply of parent tissue (p. 3). The stolonic bud is known in *Polyzoa vesiculiphora* (Watanabe and Tokioka, 1972; Fujimoto and Watanabe, 1976), but there are the following differences between *Polyzoa* and the present new species. In *Polyzoa* the stolon is attached to the substratum by some points on the ventral surface arranged like stepping-stone, whereas in the present new species it is attached to the substratum with its whole ventral surface. In *Polyzoa,* there is a test-vessel running proximo-distally beneath the stolon, whereas in the present new species no such test-vessel is seen under any stolon.

It has been generally believed that the protostigmata are possessed only by oozooids derived from fertilized eggs. Appearance of transversely elongated stigmata in blastozooids is recorded in *Stolonica* (Sélys-Longchamps, 1917) and *Metandrocarpa* (Abbott, 1953; Kawamura and Watanabe, 1981, Figs. 1 and 2). According to Sélys-Longchamps, a few transversely elongated "protostigmata" are found in the posterior region of the branchial sac in *S. socialis*, and they undergo the division by pinching into halves. In *M. taylori* and *M. uedai*, a few posterior stigmata, originally ordinary in size and shape, become elongated transversely like the protostigmata. Thus, it is shown for the first time in the present new species that the blastozooids are provided with the protostigmata at early developmental stage.

The protostigmata are also found in *P. misakiensis*, in which they are successively divided completely to produce the ordinary stigmata. In the present new species, however, transversely elongated stigmata are formed together with a small number of longitudinally elongated ones, perhaps by incomplete successive division. This feature is similar to that of *P. transversalis* Tokioka, 1963. According to Tokioka, the usual elliptical stigmata are limited to the narrow area along each side of the endostyle and in the anterior part of the area between the third and fourth branchial plications. He states that "this new species may be accepted as a form showing an intermediate stage in the process of formation of transversely elongated stigmata". However, judging from the similarity in the arrangement of stigmata between *P. transversalis* and the present new species, it is very probable that the blastozooid of *P. transversalis* is provided also with protostigmata at the early developmental stage and that the stigmatal arrangement in that species represents a transitional stage from the state of the protostigmata to that of the ordinary stigmata, or the final stage of successive divisions, brought about by incomplete successive divisions of protostigmata as seen in the present new species.

As mentioned above, the present generic identification was made on the feature of the orifices and the existence of protostigmata in blastozooids, both are shared by the present new species and *Polyandrocarpa misakiensis*. But, it will require further crucial studies in many species of *Polyandrocarpa* inclusive of *Eusynstyela, Polycarpa, Stolonica, Distomus*, etc., till the above-mentioned features are accepted truly as the generic criteria. Non-appearance of the gonad in the present new species for 27 months is very significant, but it is quite unknown whether or not this is a regular phenomenon. And then, further supposition as to the close affinity between the present species and *P. transversalis* is still prevented by this. The new species is named after its stolonic bud formation.

Summary

1. Colonies of a strange polystyelid ascidian have been cultured for 27 months. Although no gonads appeared during the observations, this ascidian is described here provisionally as a new species of *Polyandrocarpa*, as it shares some very significant characters with *P. misakiensis*.

2. There are 15–18 orifices, leading to the test-vessel, on the ventral side near the basal margin in adult zooids, but no net-work of test-vessels is found beneath the zooid. This feature resembles that in *P. misakiensis*.
3. Four to five stolons are protruded at a time from the parent mantle wall, and usually 1 to 3 stolonic buds are formed by strobilation.
4. Protostigmata appear first on the plain wall of the branchial sac of developing buds as in *P. misakiensis*. They are divided successively by formation of branchial folds and further subdivided in places to represent a "composite" arrangement of transversely elongated stigmata and a small number of longitudinally elongated ones.

REFERENCES

Abott, D.P. 1953. Asexual reproduction in the colonial ascidian *Metandrocarpa taylori* Huntsman. Univ. Calif. Publ. Zool., **61**: 1–78.

Berrill, N.J. 1948. The gonad, larvae, and budding of the polystyelid ascidian *Stolonica* and *Distomus*. J. Mar. Biol. Ass. U. K., **27**: 633–650.

Fujimoto, M., and H. Watanabe 1976. Studies on the asexual reproduction in the polystyelid ascidian, *Polyzoa vesiculiphora*. J. Morph., **150**: 607–622.

Izzard, C.S. 1973. Development of polarity and bilateral asymmetry in the palleal bud of *Botryllus schlosseri* (Pallas). J. Morph., **139**: 1–26.

Kawamura, K., and M. Nakauchi 1976. Budding and life history of the ascidian, *Symplegma viride* (in Japanese with English abstract). Rep. Usa Mar. Biol. Stat., **23**: 1–10.

Kawamura, K., and H. Watanabe 1981. Budding and test vessel system in the polystyelid ascidian, *Metandrocarpa uedai*. Mem. Fac. Kochi Univ. Ser. D, 2: 19–27.

Mukai, H., Sugimoto, K., and Y. Taneda 1978. Comparative studies on the circulatory system of the compound ascidian, *Botryllus, Botrylloides* and *Symplegma*. J. Morph., **157**: 49–78.

Newberry, A.T. 1965. Vascular structure associated with budding in the polystyelid ascidian *Metandrocarpa taylori*. Ann. Soc. Roy. Zool. Belg., **95**: 57–74.

Sélys-Longchamps, M. de 1917. Sur le bourgeonnement des Polystielines *Stolonica* et *Heterocarpa* avec quelques notes sur l'anatomie de ces deux genres. Bull. Sci. Fr. Belg., **50**: 170–276.

Sugimoto, K., and M. Nakauchi 1974. Budding, sexual reproduction and degeneration in the colonial ascidian, *Symplegma reptans*. Biol. Bull., **147**: 213–226.

Tokioka, T. 1953. Ascidians of Sagami Bay. Iwanami Shoten, Tokyo, 315 pp.

———— 1963. A new remarkable synstyelid, *Polyandrocarpa* (*Eusynstyela*) *transversalis* n. sp., from Hawaii. Publ. Seto Mar. Biol. Lab., **11**: 101–105.

Watanabe, H. 1953. Studies on the regulation in fused colonies in *Botryllus primigenus* (Ascidiae Compositae). Sci. Rep. Tokyo Kyoiku Daigaku, Sec. B, **10**: 253–284.

Watanabe, H., and T. Tokioka 1972. Two new species and one possibly new race of social styelids from Sagami Bay, with remarks on their life history, especially the mode of budding. Publ. Seto Mar. Biol. Lab., **19**: 327–345.